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Allozyme Variation in a Colonizing Species: The Cabbage Butterfly *Pieris rapae* (Pieridae)

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Abstract. Geographic patterns of variation at 7 allozyme loci in the butterfly *Pieris rapae* agree well with those expected from a knowledge of the open population structure of the insect. In the eastern United States, where the species has existed for a longer time and where there is probably more gene flow among populations, *P. rapae* populations show little or no genetic divergence. The results of this study and of our study of population structure indicate that effective population sizes are large and that the population of the entire eastern seaboard may even approach a single, panmictic unit. In the West, areas of suitable habitat in residential or agricultural areas are separated by extensive, inhospitable areas. Populations there have apparently diverged, at least slightly, from those in the East and among themselves. Although the action of other factors, especially selection, cannot be completely ruled out, it is likely that extensive gene flow among populations is the most important force in determining the overwhelming pattern of allozyme uniformity in *P. rapae*.

Introduction

The theory relating gene frequencies to elements of population structure (*sensu* Ehrlich, Holm and Parnell, 1974) such as the size and dynamics of populations is sophisticated and constantly growing more so. Although many aspects of this theory have been explored experimentally in laboratory populations, especially of *Drosophila*, there are few data from natural populations. Among numerous recent studies using allozyme methods to investigate genic variation in natural populations (see Lewontin, 1974; Powell, 1975; Nevo, 1978; and Hamrick *et al.*, 1979; for recent reviews), only a few have included an appreciable ecological compenent or have dealt with populations whose structure had been previously well characterized (McKechnie, Ehrlich and White, 1975; Schrier *et al.*, 1977; Hedgecock, 1978; Eanes and Koehn, 1979); yet these are the kinds of investigations that must be carried out if we seek correlation between elements of population structure and patterns of genetic variation.

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Allozyme methods reveal only a portion of the true allelic variation at structural loci. Nonetheless, if a number of loci are studied and if some care is taken to affirm the underlying genetic basis of the observed variation in electromorphs (*i.e.* protein variants of a single locus with different electrophoretic mobilities), these methods can provide more information on patterns of geneic variation than can most classical methods.

In this paper we report a study of allozyme variation in North American populations of the European Cabbage Butterfly, *Pieris rapae* (Linnaeus). An intensive study of adult population ecology accompanied the genetic investigations, and the results of that study will be reported in detail elsewhere. These studies were intended to provide quantitative data on population geometry, density and dispersal as well as on patterns of allozyme variation.

The Organism

Butterflies have long been important subjects in ecological genetics (Ford, 1971; Ehrlich *et al.*, 1975). Although our knowledge of the ecology of butterfly populations is more advanced than our knowledge of their genetics, there is a body of data on the interaction of population structure and genetic variation (based on phenetic characters) going back to the early work of E. B. Ford and others (Ford and Ford, 1930; Ford, 1971). The use of allozyme methods promises to improve our understanding of the genetics of butterfly populations and to allow a more satisfying synthesis than previously possible (Ehrlich *et al.*, 1975; Ehrlich and White, 1980).

Pieris rapae, known in Britain as the small garden white and in North America as the European cabbage butterfly, can aptly be labeled a "colonizing species." Its colonizing ability is perhaps best illustrated by the history of the species' rapid spread following its introduction to North America. It was introduced in Quebec around 1860 (Scudder, 1887) and had spread as far as the California coast by the 1920's (Comstock, 1927), although it may have arrived there by a much earlier, separate introduction (Emmel and Emmel, 1973). Emmel (1975) reports that *P. rapae* spread throughout Australia in the five years following its introduction to that continent in Victoria in 1939.

The larvae feed on crucifers and can be serious pests on crop plants of that family. Within its range, *P. rapae* is ubiquitous in gardens and other areas containing wild or cultivated Cruciferae. It also inhabits other temporally unstable habitats in very early stages of secondary succession or those subject to persistent disturbance.

Our mark-recapture studies of the adult population ecology of *P. rapae* in central New York State (Vawter, in prep.) demonstrated that individuals readily move up to several kilometers between areas of suitable habitat,

Fig. 1. Collection Sites, United States.

and that areas of concentration exchange individuals frequently. Various indices of vagility indicated that females have more of a tendency to disperse than males, and since virtually every female is inseminated shortly after emergence from the pupa, such dispersal almost certainly represents gene flow. At least some European populations of *P. rapae* are migratory.

Methods

Collections for this study were made between 1973 and 1976 in the areas shown in Figures 1 and 2. Most of the collecting sites were agricultural fields either currently under cultivation or in various stages of secondary succession. Three of the Tompkins Co., N.Y., sites (White Church, Belle School and Caroline Depot) lie in the Willseyville Valley, in which we carried out an intensive mark-recapture program to study the adult population structure of *P. rapae*.

All sampling was done by netting. Captured specimens were placed live in glassine envelopes and either returned immediately to the laboratory (Tompkins Co. samples) or frozen and kept on dry ice. Electrophoretic techniques were similar to those used by Selander *et al.* (1971), modified for Lepidoptera as in Vawter and Brussard (1975). We originally surveyed 24 genetic loci; most were monomorphic or only weakly polymorphic. Thus, this analysis concentrates on four highly polymorphic ones (GOT-1,

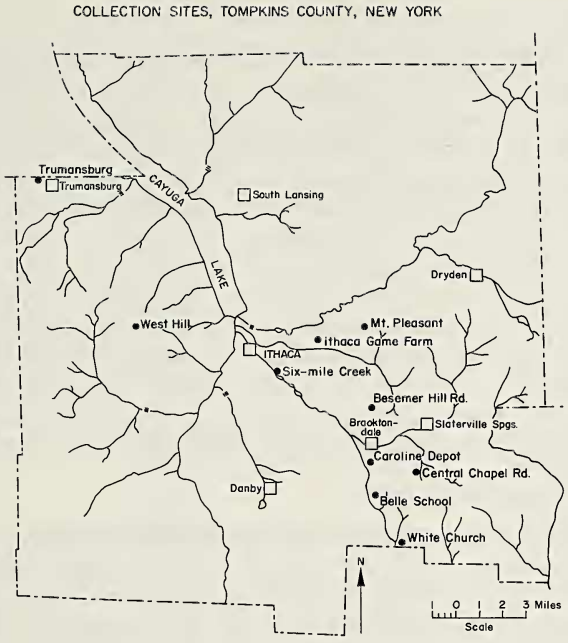

Fig. 2. Collection Sites, Tompkins County, New York.

PHI, PGM and MDH-1), and on three other slightly polymorphic loci which were clearly resolved on the same gels (MDH-2, GOT-2 and α-GPDH).

Allelic frequencies were derived directly from the observed zygotic frequencies. After lumping some electrophoretic classes to achieve appropriately large expected frequencies, these were compared to those expected under Hardy-Weinberg equilibrium. Out of 55 possible comparisons, only one sample deviated significantly from Hardy-Weinberg expectation. In order further to confirm the genetic basis for the phenotypes observed on our gels, we carried out a laboratory breeding study as well. In no case did we discover a phenotype in the F_1 which could not be explained by the observed parental phenotypes; furthermore in only one case out of 45 were the observed ratios significantly different from those expected for Mendelian inheritance of single-locus characters. These results, plus the concordance of the observed electrophoretic patterns at these loci to those known in other organisms, provide convincing evidence of the underlying genetic basis of polymorphisms. No significant differences in electromorph frequencies were ever observed between sexes, and males and females were considered together for further analysis.

Results

In cases where migration between neighboring demes is restricted, genetic differentiation on a local scale can be substantial. Although one might not, on first consideration, expect to find such patterns in populations of butterflies or other organisms capable of rapid dispersal by flight, the population structures that have been described for a number of butterfly species suggest the possibility of genetic differentiation among demes on a highly local scale (Ehrlich, 1965; Ford, 1971; Ehrlich and Gilbert, 1973; Ehrlich and Raven, 1969). To date, however, studies of genetic variation among local populations of butterflies, even those known to exchange very few individuals, have failed to reveal significant microgeographic differentiation either in phenetic (Ehrlich and Mason, 1966; Ehrlich *et al.*, 1975) or electrophoretic characters (Burns and Johnson, 1971; Ehrlich *et al.*, 1975; McKechnie *et al.*, 1975; Brussard and Vawter, 1975; Vawter and Brussard, 1975; Schrier *et al.*, 1976).

Variation on a Local Scale

We found little evidence for microgeographic differentiation at allozyme loci among the 10 areas we sampled in Tompkins Co., N.Y., between 1973 and 1976 (Tables 1 and 2). The slightly polymorphic loci were fixed or nearly fixed for the same electromorph, and frequencies at the four polymorphic loci were similar in all populations. G-tests for heterogeneity revealed no significant variation in allelic frequencies among the three broods of *P. rapae* flying in Tompkins Co. during 1974 at GOT-1, PHI and PGM, nor at the slightly polymorphic loci. At the MDH-1 locus, however, a significant heterogeneity was detected, due to a high frequency of the MDH-1a electromorph in Brood 1. This difference disappeared in

Table 1. Frequency of most common electromorph at localities within Tompkins Co., NY, 1973-1976

Locality & Date	Sample Size	GOT-1a	GOT-2b	PHIc	PGMb	αGPDHα	MDH-1b	MDH-2b
Wilseyville Valley 1973								
White Church	114	0.87	1.00	0.78	0.72	0.95	0.91	0.98
Belle School	107	0.86	0.99	0.71	0.69	1.00	0.93	0.99
Trumansburg 1973	27	0.85	—	0.74	0.70	—	0.96	1.00
Ithaca Game Farm 1973	32	0.84	—	0.68	0.75	1.00	0.93	0.97
Besemer Hill Rd. 1973	50	0.87	1.00	0.73	0.77	1.00	0.90	0.98
Wilseyville Valley 1974	266	0.83	0.99	0.74	0.74	1.00	0.92	0.90
Central Chapel Rd. 1974	34	0.88	0.98	0.68	0.74	1.00	0.90	1.00
Six-mile Creek 1974	24	0.94	0.98	0.88	0.90	1.00	0.87	1.00
West Hill 1974	21	0.90	0.96	0.76	0.75	1.00	1.00	—
Wilseyville Valley 1975	46	0.89	1.00	0.80	0.75	1.00	0.88	1.00
Mt. Pleasant 1976	50	0.87	1.00	0.69	0.64	1.00	0.94	0.98

Table 2. Heterogeneity of allozyme frequencies among localities in Tompkins Co., NY. The localities sampled each year are listed below.

	G	df	P
1973[1]			
GOT-1	2.78	8	>0.9
PHI	15.73	16	>0.1
PGM	17.14	16	>0.1
MDH-1	2.78	4	>0.5
1974[2]			
GOT-1	9.50 (5.17)	4 (3)	>0.025 (>0.1)
PHI	23.08 (13.50)	12 (9)	>0.025 (>0.1)
PGM	19.84	16	>0.1
MDH-1	3.02	4	>0.5
1973, 1974, 1976[3]			
GOT-1	7.07	7	>0.1
PHI	31.63	21	>0.05
PGM	34.08	28	>0.1
MDH-1	5.35	7	>0.5

[1]White Church, Belle School, Besemer Hill Road, Trumansburg, Ithaca Game Farm.
[2]Belle School, Caroline Depot, Six-Mile Creek, Central Chapel Road, West Hill. Values in parentheses were calculated w/o Six-mile Creek.
[3]Trumansburg, Ithaca Game Farm, Besemer Hill Road (1973); Wilseyville Valley combined, Central Chapel Road, Six-mile Creek, West Hill (1974); Mt. Pleasant (1976).

subsequent years, however. Finally, G-tests revealed no heterogeneity among allelic frequencies at any locus among years (1973-1975) in the Willseyville Valley. Thus, samples taken anywhere within the valley were pooled for comparison with other areas. Table 2 shows the results of heterogeneity tests among Tompkins Co. localities. For 1973 we detected heterogeneities at two of the four polymorphic loci.

Examination of the 1974 electromorph frequencies themselves indicated that the Six-mile Creek population was aberrant at both GOT-1 and PHI, and perhaps at MDH-1 (Table 1), and when this population was removed from the comparison, the heterogeneity disappeared. The heterogeneity observed when the Six-mile Creek population was included, although significant at the 0.05 level, was not striking; and, given the relatively small sample size from this population (N=24), it may merely represent sampling error.

With the possible exception of Six-mile Creek, therefore, the overall pattern of genetic variation at the local level for *P. rapae* is one of genetic constancy without significant microgeographic differentiation. When we compared electromorph frequencies among all of the Tompkins Co. localities regardless of year of sampling (Table 1), we found no significant heterogeneities. That is to say, there is no genetic evidence which suggests that *P. rapae* in Tompkins Co. do not represent a single, essentially panmictic population.

Variation on a Continental Scale

In addition to the Tompkins Co. sites, we collected allozyme data from 14 additional sites that span most of the range of *P. rapae* in North America (Table 3, Figure 1). There is a striking pattern of genetic

Table 3. Frequency of most common electromorph at localities outside of Tompkins Co., N.Y., various years.

Locality	Sample Size	GOT-1a	GOT-2b	PHIc	PGMb	αGPDHa	MDH-1b	MDH-2b
Stillwater Reservoir, NY	38	0.90	1.00	0.76	0.68	0.99	0.96	—
New Boston, NY	38	0.89	0.99	0.74	0.64	1.00	0.96	—
Appledore Island, NH	20	0.92	1.00	0.80	0.63	1.00	0.93	1.00
Liverpool, PA	28	0.91	1.00	0.80	0.70	1.00	0.90	1.00
Salisbury, MD	33	0.82	1.00	0.77	0.67	1.00	0.93	1.00
Chincoteague, VA	41	0.82	1.00	0.82	0.58	1.00	0.83	—
Mauzy, VA	44	0.81	0.99	0.83	0.74	1.00	0.91	1.00
Saluda, VA	72	0.84	1.00	0.78	0.70	1.00	0.90	0.98
Clinton, NC	29	0.88	1.00	0.67	0.68	1.00	0.93	1.00
Escambia, AL	46	0.97	0.99	0.83	0.82	1.00	0.91	0.99
Lincoln, NB	34	0.91	1.00	0.64	0.90	—	1.00	1.00
Reno, NV	92	0.96	1.00	0.80	0.68	1.00	0.92	1.00
Beaverton, OR	52	0.88	1.00	0.85	0.73	1.00	0.87	1.00
Arcadia, CA	82	0.88	1.00	0.84	0.87	—	0.93	0.99

constancy over this area as well. At the four slightly polymorphic loci, the same electromorph is fixed or nearly so in all populations, and frequencies at the polymorphic loci are similar throughout. Although populations in the western United States (Lincoln, Neb.; Beaverton, Ore.; Reno, Nev.; Arcadia, Calif.) and near the southern periphery of the species' range (Escambia Co., Ala.) show some noticeable differences, the same electromorph predominates in all populations, and the order of electromorphs from most to least common is nearly the same. The apparent fixation of the MDH-1b electromorph at Lincoln, Neb., may be a result of the small sample size in this case.

The similarity among the eastern U.S. populations is well illustrated by the results of G-tests of heterogeneity (Table 4) performed on the number of genes sampled at each of the four polymorphic loci (2N). For three of the four loci (GOT-1, PHI and MDH-1), there are no significant differences among 10 localities ranging from northern New York State to central

Table 4. Heterogeneity of allozyme frequencies among eastern U.S. localities. Localities include: Stillwater Reservoir, NY; New Boston, NY; Wilseyville Valley, NY (1974); Appledore Island, NH; Liverpool, PA; Salisbury, MD; Chincoteague, VA; Mauzy, VA; Saluda, VA; Clinton, NC; and Escambia Co., AL.

	G	df	P
Without Escambia Co., AL			
GOT-1	9.76	9	>0.1
PHI	26.99	27	>0.1
PGM	41.27	27	>0.025
MDH-1	13.09	9	>0.05
With Escambia Co., AL			
GOT-1	23.62	10	>0.01
PHI	31.47	30	>0.1
PGM	52.88	30	>0.005
MDH-1	13.16	10	>0.05

North Carolina, while for the fourth locus (PGM) the heterogeneity among these localities is significant and due primarily to the contribution of a single population (Chincoteague, Va.). The Escambia Co., Ala., population is the most aberrant of the eastern U.S. populations.

The western populations are not only different from the eastern populations, they also tend to be more heterogeneous among themselves. At the GOT-1 locus, an electromorph (a') which does not occur in any eastern population occurs at very low frequency at both Lincoln, Neb., and Beaverton, Ore.; but in general, the difference between the western and eastern populations tends to be the absence in the west of electromorphs that are rare in the east.

There is, then, a striking pattern of uniformity in allozyme frequencies among populations. Eastern populations from New York to North Carolina form a homogeneous unit. However, geographically peripheral populations tend to diverge from this pattern. We made pair-wise comparisons based on all 7 loci between all populations using the index of genetic identity described by Nei (1972) for which a value of 1.00 indicates

complete allelic identity (*i.e.* all alleles shared in identical frequencies). In hopes of identifying clusters of populations, we performed a Bray-Curtis ordination on two axes, using arcsine transforms of the genetic distances (see Brussard, 1975, for a description of this technique). The results of this ordination are shown in Figure 3. We chose the two genetically most dissimilar sites, Lincoln, Neb., and Chincoteague, Va., (I=.973), as the end-points of the first axis, and Six-mile Creek, N.Y., and Clinton, N.C., (I=.988) as the end-points of the second axis. Some degree of clustering

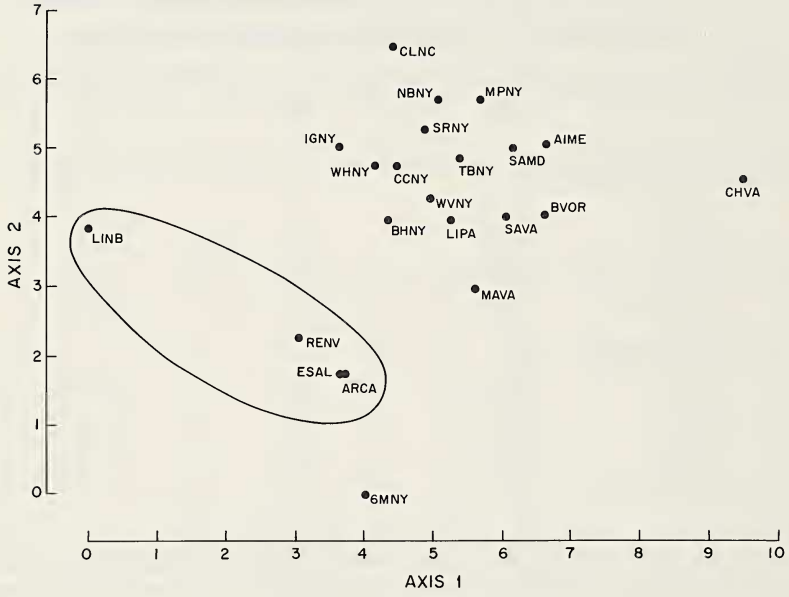

Fig. 3. 2-axis Bray-Curtis plot on arcsine transforms of genetic distance. Based on frequencies of 18 electromorphs in 22 populations. Note the tendency for geographically peripheral populations to diverge from most of the populations of the eastern United States. Beaverton, Oregon, is an exception.

AIME, Appledore Island, ME
ARCA, Arcadia, CA
BHNY, Besemer Hill, NY
BVOR, Beaverton, OR
CCNY, Central Chapel Rd., NY
CHVA, Chincoteague, VA
CLNC, Clinton, NC
ESAL, Escambia Co., AL
IGNY, Ithaca Game Farm, NY
LINB, Lincoln, NB
LIPA, Liverpool, PA
MAVA, Mauzy, VA
MPNY, Mount Pleasant, NY
NBNY, New Boston, NY
RENV, Reno, NV
SAMD, Salisbury, MD
SAVA, Saluda, VA
SRNY, Stillwater Res., NY
TBNY, Trumansburg, NY
WHNY, West Hill, NY
WVNY, Wilseyville Valley, NY
6MNY, Six-mile Creek, NY

was obtained by this analysis, indicating the central-peripheral dichotomy mentioned earlier. Two of the western populations (Reno, Nev., and Arcadia, Calif.) cluster with Escambia Co., Ala., in the lower left of the plot, but another western population (Beaverton, Ore.) is distant from these. Neither of the axes appears to parallel latitude or longitude, and geographically close sites are frequently quite distant from each other in this ordination. Another ordination based on Roger's (1972) Coefficient of Genetic Similarity without transformation, done using the Cornell Ecology Program Series (CEP-4, Gauch, 1973) produced nearly identical results, indicating that the patterns were not an artifact of the distance measure or transformation chosen. A 3-axis principal components plot based directly on the electromorph frequencies (Fig. 4) shares many similarities with the

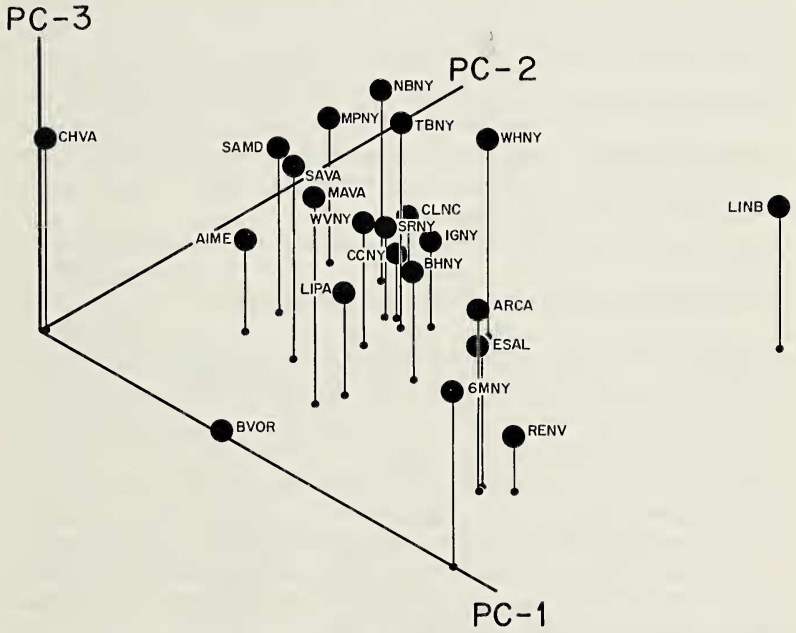

Fig. 4. 3-axis principal component plot on electromorph frequencies. Based on the same data as Figure 3. Note that geographically peripheral populations are separated from most eastern U. S. populations in this plot. Beaverton, OR, although similar to eastern U. S. populations on PC axis 1, is separated from them on axes 2 and 3. Abbreviations are the same as in Figure 3.

Bray-Curtis plots; the clusters are similar, and the extreme populations are the same, although Beaverton, Ore., now appears with other peripheral populations. The similarity between the aberrant Six-mile Creek sample and geographically peripheral populations is also well illustrated.

Discussion

In any species that readily colonizes disturbed, temporally unstable habitats, two processes tend to work against each other to determine genetic patterns among and within populations. On the one hand, to take advantage of newly available areas for colonization, such species must be relatively vagile and have a considerable dispersal ability. From our study of the population structure of *Pieris rapae*, it appears that this species fits this requirement well, and one would expect that frequent movements among neighboring populations and occasional long-distance migration would tend to swamp any tendency toward interpopulation differentiation. On the other hand, if newly available areas are colonized by a small number of founders and if population size tends to fluctuate drastically, as one might expect it to do in temporally unstable habitats, one would expect to find a considerable degree of genetic variation among populations. Within such populations, many of which must be marginal in an ecological sense, low effective population size, founder effects, and inbreeding should also tend to reduce within-population variation (Soulé, 1973). Geographic patterns of allozyme variation in *P. rapae* reflect the action of both of these processes operating at different intensities in different parts of the species' North American range.

In a study of variation at 11 allozyme loci in the checkerspot butterfly, *Chlosyne palla* Boisduval, Schrier *et al.* (1976) found no significant differences in gene frequencies among areas separated by up to 12 km. In the same study, these authors demonstrated an "open" structure for populations of *C. palla*, and suggest that this pattern of movement and the resultant geographic uniformity of allozyme frequencies was correlated with foodplant distribution. The foodplants of *P. rapae* are very widespread; they are also largely ephemeral. This fact may account for the population structure observed in this colonizing species and the lack of significant genic difference among populations separated by thousands of km. Frequent population extinction and recolonization in weedy or otherwise ephemeral habitats have been evoked as an explanation for lack of genetic differences among populations of other species of butterflies (Shapiro, 1974; Brussard and Vawter, 1975).

Wilson (1965), citing Wallace (1959) and Lewontin (1961), argues that the best measure of population fitness is not the average of the relative fitness of individual genotypes, but the length of time the population persists. The application of this argument to *P. rapae* or to species with similar population structures is not straightforward. Populations in a demographic sense, as aggregations of organisms, are short-lived; however, because of extensive movements among areas of concentration and the resultant genetic uniformity, the founding and extinction of such areas within the species' range may have only minor genetic and evolutionary consequences.

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